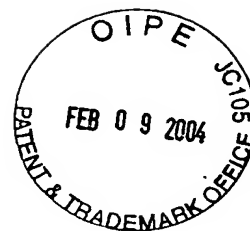


SEQUENCE LISTING



<110> Li, Henry
Chatterton, Jon E.
Ke, Ning
Rhoades, Kristina L.
Wong-Staal, Flossie
Immusol Inc.

<120> Single Promoter System for Making siRNA Expression
Cassettes and Expression Libraries Using a Polymerase
Primer Hairpin Linker

<130> 016556-003210US

<140> US 10/628,587

<141> 2003-07-23

<150> US 60/399,040

<151> 2002-07-24

<160> 34

<170> PatentIn Ver. 2.1

<210> 1

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:upstream primer
Hind III U6-265 modified to contain a Hind III
site outside the 5' end of the U6 promoter

<400> 1

tgctaagctt aaggtcgggc aggaagag

28

<210> 2

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:downstream
primer S-U6-20 modified to contain a Sph I
restriction site at the 3' end of the U6 promoter

<400> 2

atcgcatgc agatatataa agccaa

26

<210> 3

<211> 43

<212> DNA

<213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:chemically
 synthesized self-priming oligonucleotide siRNA-LIBh
 with complement of pol III promoter type III
 termination signal, randomized "sense" coding
 sequence for hairpin siRNA and linker and primer for
 synthesis of "antisense" strand

<220>
 <221> modified_base
 <222> (1)
 <223> n = 5' phosphorylated c

<220>
 <221> modified_base
 <222> (16)..(33)
 <223> n = g, a, c or t

<400> 3
 ngaccactct aaaaannnnn nnnnnnnnnn nnnngcgttcg cgc 43

<210> 4
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:chemically
 synthesized universal oligonucleotide Univ-1h (Sph
 I)

<400> 4
 tttttagagt ggtcgcgtg 19

<210> 5
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:chemically
 synthesized universal oligonucleotide Univ-2h (Bam
 HI)

<220>
 <221> modified_base
 <222> (1)
 <223> n = 5' phosphorylated g

<400> 5
 natccgacct ctctaaaaa 19

<210> 6
 <211> 43
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:chemically
 synthesized oligonucleotide siRNAh-lucB

<220>
 <221> modified_base
 <222> (1)
 <223> n = 5' phosphorylated c

<400> 6
 ngaccactct aaaaagtgcg ctgctggtgc caacccttcg ggg 43

<210> 7
 <211> 43
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:chemically
 synthesized oligonucleotide siRNAh-SCRAMBLE

<220>
 <221> modified_base
 <222> (1)
 <223> n = 5' phosphorylated c

<400> 7
 ngaccactct aaaaagcgcg ctttgtagga ttcgcgttcg cgc 43

<210> 8
 <211> 54
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:downstream
 primer S-U6-TET-o modified to contain a Sph I
 restriction site at the 3' end of the U6 promoter
 and incorporating tetracycline operator sequence

<400> 8
 atcggcatgc agatatataa ctctatcaat gatagagtac tttcaagtta cggt 54

<210> 9
 <211> 97
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:self-priming
 oligonucleotide HpLib containing spacer, Asc I
 restriction site, part of XmaI site, spacer,
 complement to transcription terminator, randomized
 siRNA coding sequence and polymerase primer hairpin
 linker

<220>
 <221> modified_base
 <222> (31)..(49)
 <223> n = g, a, c or t, randomized siRNA coding sequence

<400> 9
 ttctagagggc ggcggggcc gccaaaaaag nnnnnnnnnn nnnnnnnnnc ttcaagcgaa 60
 gagcgctcc gggtacggag gcgctcttcg aagagag 97

<210> 10
 <211> 32
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:2nd Strand
 Primer

<400> 10
 ccccccccc ccccccggg ccgcaaaaa ag 32

<210> 11
 <211> 42
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:self-priming
 oligonucleotide with 5' leader sequence,
 randomized siRNA coding sequence and polymerase
 primer hairpin linker sequence

<220>
 <221> modified_base
 <222> (7)..(10)
 <223> n = g, a, c or t

<220>
 <221> modified_base
 <222> (16)..(32)
 <223> n = g, a, c or t, coding sequence for "sense"
 strand of siRNA

<400> 11
 ggccgcnnnn aaaaannnnn nnnnnnnnnn nngggttcgc cc 42

<210> 12
 <211> 74
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:self-priming
 oligonucleotide after primer extension of SEQ ID
 NO:11 to generate sequence complementary to 5'
 leader sequence and randomized siRNA coding
 region "sense" strand to form a stem-loop structure

<220>
 <221> modified_base
 <222> (7)..(10)
 <223> n = g, a, c or t, complementary to n = g, a, c or
 t at positions 68-65

<220>
 <221> modified_base
 <222> (16)..(32)
 <223> n = g, a, c or t, complementary to n = g, a, c or
 t at positions 59-43

<220>
 <221> modified_base
 <222> (43)..(59)
 <223> n = g, a, c or t, complementary to n = g, a, c or
 t at positions 32-16

<220>
 <221> modified_base
 <222> (65)..(68)
 <223> n = g, a, c or t, complementary to n = g, a, c or
 t at positions 10-7

<400> 12
 ggccgcnnnn aaaaannnnn nnnnnnnnnn nnggggttcgc ccnnnnnnnnn nnnnnnnnnnt 60
 ttttnnnngc ggcc 74

<210> 13
 <211> 11
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:5' blocking
 primer

<220>
 <221> modified_base
 <222> (6)..(9)
 <223> n = g, a, c or t, complementary to n = g, a, c or t
 at positions 10-7 of SEQ ID NO:12

<400> 13
 tttttnnng c 11

<210> 14
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:3' blocking
 primer

<220>
 <221> modified_base
 <222> (11)..(14)
 <223> n = g, a, c or t, complementary to n = g, a, c or t
 at positions 68-65 of SEQ ID NO:12

<400> 14
 cgcgggccgc nnnnaaaaaa 19

<210> 15
 <211> 74
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:completed
 expression cassette sequence with synthesized
 segment complementary to single stranded region
 between blocking primers

<220>
 <221> modified_base
 <222> (11)..(14)
 <223> n = g, a, c or t, complementary to positions 72-69

<220>
 <221> modified_base
 <222> (20)..(36)
 <223> n = g, a, c or t, complementary to positions 63-47

<220>
 <221> modified_base
 <222> (47)..(63)
 <223> n = g, a, c or t, complementary to positions 36-20

<220>
 <221> modified_base
 <222> (69)..(72)
 <223> n = g, a, c or t, complementary to positions 14-11

<400> 15
 cgcgggccgc nnnnaaaaaan nnnnnnnnnnn nnnnnngggc gaaccnnnnn nnnnnnnnnnn 60
 nnnntttttnn nngc 74

<210> 16
 <211> 137
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:self-priming
 oligonucleotide HpLib after primer extension with
 reverse trtanscriptase (RT)

<220>
 <221> modified_base
 <222> (31)..(49)
 <223> n = g, a, c or t, complementary to positions 107-89

```

<220>
<221> modified_base
<222> (89)..(107)
<223> n = g, a, c or t, complementary to positions 49-31

<400> 16
ttctagaggc gcgccgggcc gccaaaaaag nnnnnnnnnn nnnnnnnnnc ttcaagcgaa 60
gagttacgga ggcgctcttc gaagagagnn nnnnnnnnnn nnnnnnnctt tttggcggc 120
ccggcgcgcc tctagaa 137

<210> 17
<211> 115
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
oligonucleotide HpLib after primer extension with
reverse trtanscriptase (RT) and AscI digestion to
yield recessed 3' end

<220>
<221> modified_base
<222> (22)..(40)
<223> n = g, a, c or t, complementary to positions 98-80

<220>
<221> modified_base
<222> (80)..(98)
<223> n = g, a, c or t, complementary to positions 40-22

<400> 17
cgcgccgggc cgccaaaaaa gnnnnnnnnn nnnnnnnnnn cttcaagcga agagttacgg 60
aggcgctctt cgaagagagn nnnnnnnnnn nnnnnnnnct ttttggcgg cccgg 115

<210> 18
<211> 118
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
oligonucleotide HpLib after primer extension with
reverse transcriptase (RT), AscI digestion to
yield recessed 3' end and addition of oligo(dG)
homopolymer tail using terminal transferase

<220>
<221> modified_base
<222> (22)..(40)
<223> n = g, a, c or t, complementary to positions 98-80

<220>
<221> modified_base
<222> (80)..(98)
<223> n = g, a, c or t, complementary to positions 40-22

<400> 18
cgcgccgggc cgccaaaaaa gnnnnnnnnn nnnnnnnnnn cttcaagcga agagttacgg 60

```

aggcgctctt cgaagagagn nnnnnnnnnn nnnnnnnnct tttttggcgg cccggggg 118

<210> 19
<211> 126
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence:self-priming
oligonucleotide HpLib after primer extension with
reverse transcriptase (RT) and AscI digestion to
yield recessed 3' end and addition of oligo(dG)
homopolymer tail using terminal transferase and
ligation of AscI linkers using T4 DNA ligase

<220>
<221> modified_base
<222> (30)..(48)
<223> n = g, a, c or t, complementary to positions 106-88

<220>
<221> modified_base
<222> (88)..(106)
<223> n = g, a, c or t, complementary to positions 48-30

<400> 19
ggcgcgccccg cgccgggccc ccaaaaaagn nnnnnnnnnn nnnnnnnnct tcaagcgaag 60
agttacggag gcgctcttcg aagagagnnn nnnnnnnnnn nnnnnncttt tttggcggcc 120
cgggggg 126

<210> 20
<211> 117
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:self-priming
oligonucleotide HpLib SEQ ID NO:19 after digestion
with AscI and XmaI to yield distinct 5' overhang

<220>
<221> modified_base
<222> (28)..(46)
<223> n = g, a, c or t, complementary to positions 104-86

<220>
<221> modified_base
<222> (86)..(104)
<223> n = g, a, c or t, complementary to positions 46-28

<400> 20
cgcgccccgc cggggccgcc aaaaaagnnn nnnnnnnnnn nnnnnncttc aagcgaagag 60
ttacggaggc gctcttcgaa gagagnnnnn nnnnnnnnnn nnnncttttt tggcggc 117

<210> 21
<211> 126
<212> DNA
<213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:self-priming
 oligonucleotide HpLib complement of SEQ ID NO:19
 after digestion with AscI and XmaI to yield
 distinct 5' overhang

<220>
 <221> modified_base
 <222> (18)..(36)
 <223> n = g, a, c or t, complementary to positions 103-85

<220>
 <221> modified_base
 <222> (85)..(103)
 <223> n = g, a, c or t, complementary to positions 36-18

<400> 21
 ccggggccgcc aaaaaaagnnn nnnnnnnnnn nnnnnnctct cttcgaagag cgcctccgta 60
 accggaggcg ctcttcgctt gaagnnnnnnn nnnnnnnnnn nnnctttttt ggcggcccg 120
 cgcggg 126

<210> 22
 <211> 124
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:self-priming
 oligonucleotide HpLib, SEQ ID NO:20 AscI/XmaI
 digested product after ligation into vector
 bearing U6 snRNA promoter

<220>
 <221> modified_base
 <222> (30)..(48)
 <223> n = g, a, c or t, complementary to positions 106-88

<220>
 <221> modified_base
 <222> (88)..(106)
 <223> n = g, a, c or t, complementary to positions 48-30

<400> 22
 ggcgcgcccg cgccggggccg caaaaaagn nnnnnnnnnn nnnnnnnnct tcaagcgaag 60
 agttacggag gcgctcttcg aagagagnnn nnnnnnnnnn nnnnnncttt ttggcgcc 120
 cggg 124

<210> 23
 <211> 52
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:SEQ ID NO:22
 after digestion with SapI and elimination of
 majority of polymerase primer hairpin linker

<220>
 <221> modified_base
 <222> (30)..(48)
 <223> n = g, a, c or t, complementary to positions 26-8
 of SEQ ID NO:24 and positions 26-8 of SEQ ID NO:25

 <400> 23
 ggcgcgcccg cgccgggccc ccaaaaaagn nnnnnnnnnn nnnnnnnnct tc 52

 <210> 24
 <211> 44
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:SEQ ID NO:22
 after disgetion with SapI and elimination of
 majority of polymerase primer hairpin linker

 <220>
 <221> modified_base
 <222> (8)..(26)
 <223> n = g, a, c or t, complementary to positions 48-30
 of SEQ ID NO:23 and positions 37-19 of SEQ ID NO:26

 <400> 24
 aagagagnnn nnnnnnnnnn nnnnnncttt tttggcggcc cggg 44

 <210> 25
 <211> 55
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:complement of
 SEQ ID NO:22 after disgetion with SapI and
 elimination of majority of polymerase primer hairpin
 linker

 <220>
 <221> modified_base
 <222> (8)..(26)
 <223> n = g, a, c or t, complementary to positions 48-30
 of SEQ ID NO:23 and positions 37-19 of SEQ ID NO:26

 <400> 25
 cttgaagnnn nnnnnnnnnn nnnnnncttt tttggcggcc cggcgcgggc gcgcc 55

 <210> 26
 <211> 41
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:complement of
 SEQ ID NO:22 after disgetion with SapI and
 elimination of majority of polymerase primer hairpin
 linker

<220>
 <221> modified_base
 <222> (19)..(37)
 <223> n = g, a, c or t, complementary to positions 26-8
 of SEQ ID NO:24 and positions 26-8 of SEQ ID NO:25

 <400> 26
 cccgggccgc caaaaaagnn nnnnnnnnnn nnnnnnnctc t 41

 <210> 27
 <211> 96
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:intramolecular
 re-ligation of SEQ ID NOs:23 and 24 forming coding
 region for loop expressed as component of hairpin
 siRNA

 <220>
 <221> modified_base
 <222> (30)..(48)
 <223> n = g, a, c or t, complementary to positions 78-60

 <220>
 <221> modified_base
 <222> (60)..(78)
 <223> n = g, a, c or t, complementary to positions 48-30

 <400> 27
 ggcgcgcccg cgccgggccg caaaaaag nnnnnnnnnn nnnnnnnct tcaagagagn 60
 nnnnnnnnnn nnnnnnnnct ttttggcgg cccggg 96

 <210> 28
 <211> 15
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Description of Artificial Sequence:5' leader
 sequence, first segment of self-priming
 oligonucleotide

 <220>
 <221> modified_base
 <222> (7)..(10)
 <223> n = g, a, c or t

 <400> 28
 ggccgcnnnn aaaaa 15

 <210> 29
 <211> 10
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:remainder of 5'
 leader sequence before complement of transcription
 termination sequence at 3' end of leader sequence

<220>
 <221> modified_base
 <222> (7)..(10)
 <223> n = g, a, c or t

<400> 29
 ggccgcnnnn 10

<210> 30
 <211> 10
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:polymerase
 primer hairpin linker, third segment of self-priming
 oligonucleotide

<400> 30
 gggttcgccc 10

<210> 31
 <211> 15
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:segment
 complementary to 5' leader sequence

<220>
 <221> modified_base
 <222> (6)..(9)
 <223> n = g, a, c or t

<400> 31
 tttttnnnng cggcc 15

<210> 32
 <211> 10
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:linker and
 primer for synthesis of "antisense" strand of
 hairpin siRNA

<400> 32
 gcgttcgcgc 10

<210> 33
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:1-N segment of
polymerase primer hairpin linker

<400> 33
cttcaagcga agagcgcctc cg 22

<210> 34
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:3-N segment of
polymerase primer hairpin linker

<400> 34
cggaggcgct cttcgaagag ag 22